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Retrospective evaluation of routine whole genome sequencing of *Mycobacterium tuberculosis* at the Belgian National Reference Center, 2019

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ABSTRACT

Objectives: Since January 2019, the Belgian National Reference Center for Mycobacteria (NRC) has switched from conventional typing to prospective whole-genome sequencing (WGS) of all submitted *Mycobacterium tuberculosis* complex (MTB) isolates. The ISO17025 validated procedure starts with semi-automated extraction and purification of gDNA directly from the submitted MGIT tubes, without preceding subculturing. All samples are then sequenced on an Illumina MiSeq sequencer and analyzed using an in-house developed and validated bioinformatics workflow to determine the species and antimicrobial resistance. In this study, we retrospectively compare results obtained via WGS to conventional phenotypic and genotypic testing, for all Belgian MTB strains analyzed in 2019 (n = 306).

Results: In all cases, the WGS-based procedure was able to identify correctly the MTB species. Compared to MGIT drug susceptibility testing (DST), the sensitivity and specificity of genetic prediction of resistance to first-line antibiotics were respectively 100 and 99% (rifampicin, RIF), 90.5 and 100% (isoniazid, INH), 100 and 98% (ethambutol, EMB) and 61.1 and 100% (pyrazinamide, PZA). The negative predictive value was above 95% for these four first-line drugs. A positive predictive value of 100% was calculated for INH and PZA, 80% for RIF and 45% for EMB.

Conclusions: Our study confirms the effectiveness of WGS for the rapid detection of *M. tuberculosis* complex and its drug resistance profiles for first-line drugs even when working directly on MGIT tubes, and supports the introduction of this test into the routine workflow of laboratories performing tuberculosis diagnosis.

KEYWORDS

Whole-genome sequencing; tuberculosis; resistance; diagnostic; bioinformatic

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex, still represents a global public health problem with 10 million new cases of active disease and 1.2 million deaths reported in 2019 by the World Health Organization [1]. In Belgium, 8.5 cases per 100,000 inhabitants were reported in 2019 classifying it as a low TB incidence country. However, incidence peaks are observed in specific regions, such as Brussels (28.1/100,000 inhabitants in 2019) and other big cities. Approximately 4% of Belgian TB cases present resistance to one drug and 2% are multidrug-resistant (MDR) defined as resistant to isoniazid and rifampicin [2]. In the light of international migration, quick detection and efficient monitoring of MDR-TB is crucial in high-income countries. Indeed, the incidence of tuberculosis is high among migrants, and rapid detection can reduce the TB burden and the transmission of MDR-TB in migrants and the community at large [3].

Fast diagnosis, adequate treatment, and contact tracing are crucial elements to stop further transmission and control the disease. In recent years, the use of whole-genome sequencing (WGS) for TB diagnosis has increased, as it allows simultaneously subspecies identification, detection of mutations associated with drug resistance, and phylogenetic/clustering analyses to guide contact investigations [4]. In contrast to line probe assays, WGS has the potential to detect resistance for all anti-tuberculosis drugs including the detection of rare mutations, usually missed by classical molecular tests [5,6]. To this aim, various web-based tools have been developed to perform WGS-based prediction of drug resistance in MTB [5,7–9]. Most recently, the WHO published a catalogue of mutations in MTB and their association with drug resistance, assembling phenotype and WGS data on a set of >38,000 international MTB isolates [10].

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Supplemental data for this article can be accessed here.

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Consequently, a shift to solely WGS diagnostic typing has been done in the USA [11], the National Institute for Public Health and the Environment (RIVM) in the Netherlands, and at Public Health England [8,12], and is under investigation in many other developed countries including France and Finland [13,14].

In Belgium, the detection of drug resistance to first-line drugs is performed by eleven peripheral laboratories and the National Reference Centre (NRC), which all use phenotypic methods (mainly DST in MGIT® 960 system (BD Diagnostics, Sparks, MD)). Since January 2019, WGS was introduced in the NRC and ran in parallel with the conventional methods during the entire calendar year (Figure 1), including phenotypical drugs resistance evaluation by the MGIT system.

Here, we report the analysis and comparison of the drug resistance/susceptibility profiles obtained by these phenotypic (DST in MGIT) and genotypic (WGS) methods on the MTB strains submitted to both analyses in the NRC in 2019. The implementation of a specific, semi-automated DNA extraction methodology, part of the WGS workflow, is detailed in this paper. The wet and dry lab WGS analyses, which included the development of an in-house bioinformatics pipeline for data processing, have previously been described [15]. We evaluate the performance of sequence-based species identification and compare turnaround times between standard laboratory practice and WGS to evaluate the accuracy and speed of WGS-based diagnostics and to assess its capacity to eventually replace the phenotypic drug resistance detection within Belgium.

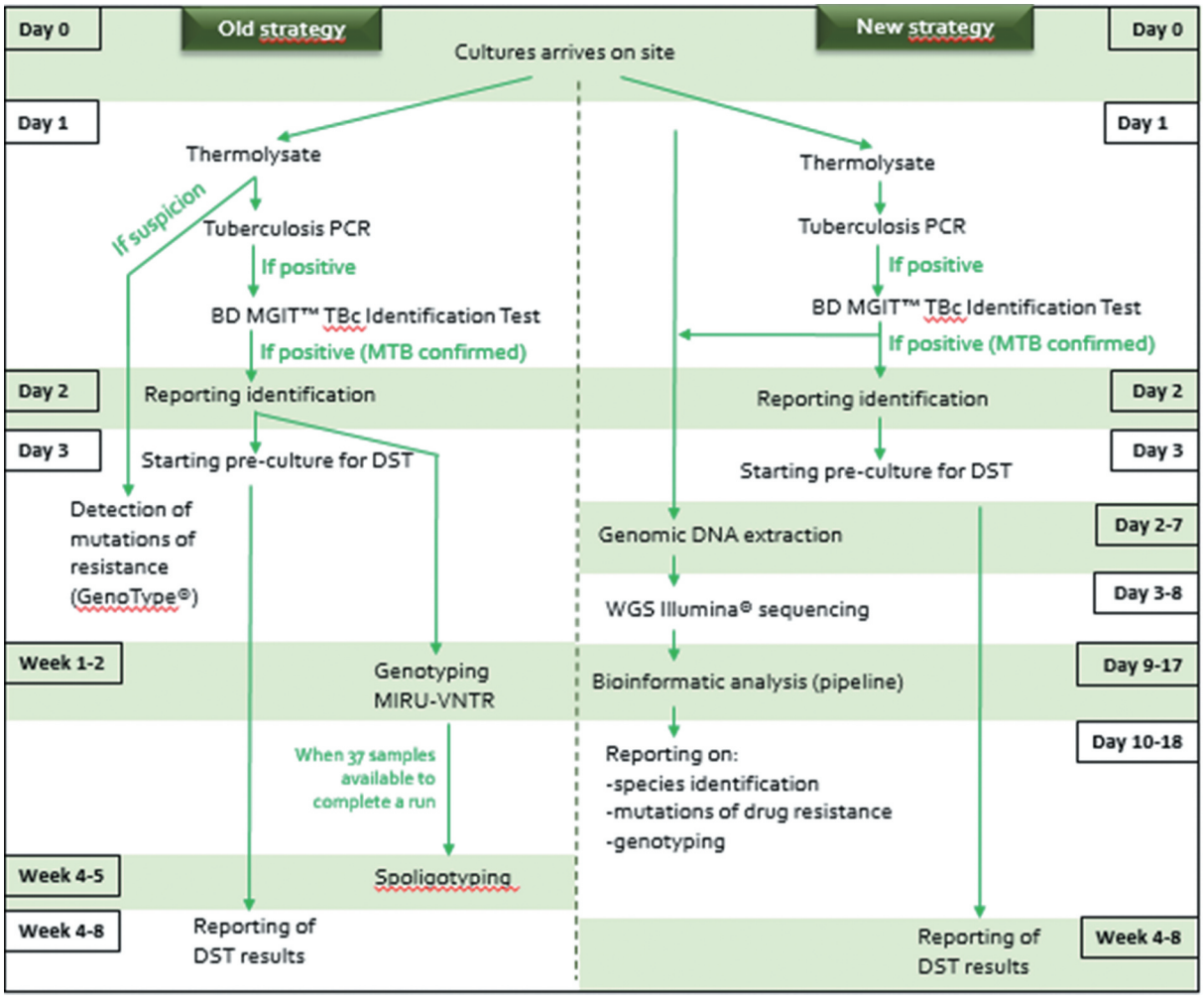

Figure 1. Comparison of the old (using conventional methods) and new (using WGS) diagnostic/surveillance strategies used in the Belgian NRC for *M. tuberculosis* complex. Turnaround time for the different steps is given. In case of urgent analysis, a GeneXpert® MTB/RIF assay can be performed. Tuberculosis PCR: Polymerase Chain reaction specific for *Mycobacterium tuberculosis* complex based on the detection of the 12.7-kb fragment [16] MTB: *Mycobacterium tuberculosis* complex DST: Drug susceptibility Testing MIRU-VNTR: Mycobacterial Interspersed Repetitive Unit Variable Number Tandem Repeat

Material and methods

Clinical isolates

This study comprises all cultures (n = 3243) received from 1st of January, 2019, to 31st of December, 2019 by the Belgian NRC for species identification, drug susceptibility testing and/or genotyping. These cultures were sent from 78 different Belgian clinical laboratories to the NRC.

Conventional species identification

Identification of *M. tuberculosis* complex was performed by multiplex PCR on the 12.7-kb fragment [16]. Confirmation of positive cases was performed using the BD MGIT™ TBc Identification Test (Becton Dickinson, Sparks, MD).

Drug susceptibility testing (DST)

DST for first-line drugs (isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA)) was performed using the BACTECT MGIT960 system (Becton-Dickinson, Sparks, MD, USA) following the manufacturer's recommendations.

Conventional molecular tests for drug resistance detection

According to the demand of the primary care laboratories or the urgency of the demand, for some strains, the line probe assay GenoType® MTBDRplus (Hain Lifescience, Nehren, Germany) was performed according to the manufacturer's recommendations. For some other strains, the GeneXpert® MTB/RIF ASSAY (Cepheid, Sunnyvale, CA, USA) was used to detect resistance to rifampicin.

DNA extraction methodology

A volume of 1 mL of the identified mycobacterial culture was inactivated 15 minutes at 95°C and treated by bead-beating 3 × 30 sec with 0.5 mm Zirconia/Silica beads (Biospec) using the Mini-Bead-Beater-16 (Biospec). The pellet collected after centrifugation (2 min at 13,000 g) was used for the extraction outside the BSL3 lab with the MagCore® Genomic DNA Bacterial kit to extract genomic DNA (gDNA) via the MagCore® auto-extraction instrument. The associated kit contains all required reagents and labware for automated purification using the magnetic particle technology. After extraction, the amount of DNA was quantified by the Quantus™ Fluorometer, allowing the sensitive detection of nucleic acids (detection limit of 10 pg/µl) using QuantiFluor® dye systems (QuantiFluor® dsDNA system).

Whole-Genome Sequencing (WGS) and data analysis

WGS was performed using an ISO17025 accredited workflow and was previously described [15]. Briefly, Nextera XT libraries (Illumina, San Diego, CA) were constructed with a 15-cycle PCR indexing step (Shea et al., 2017). One ng of MTB gDNA was used as input for tagmentation, unless this amount was not available in which case, 5 µl of the genomic DNA extract was used. Libraries were pooled and subsequently sequenced on a MiSeq instrument (Illumina, San Diego, CA) using the MiSeq V3 chemistry, following the manufacturer's instructions, for the production of 2 × 250 bp paired-end reads. The bioinformatics workflow is described in Bogaerts et al. [15] and briefly summarized here. Reads are first quality trimmed and then *de novo* assembled into contigs and mapped against the H37Rv reference genome. Various quality control metrics are then evaluated to determine if the data quality is sufficient for running the workflow. Kraken analysis is performed in order to detect presence of contaminants. SNPs and indels are called using SAMtools and BCFtools and compared against an in-house database constructed from the literature to predict resistance or susceptibility to various drugs [17,18]. Subspecies identification is performed using a combination of genomic markers including *hsp65*, the *csb/*regions of difference (RD) classification, 16S rRNA, and lineage-defining SNPs [19].

For each detected mutation, the workflow reports the known associations with antimicrobial resistance or susceptibility (i.e. entries in the in-house database). When a mutation is detected that is not present in the database, the workflow reports an 'unknown' association to the drug(s) associated with the corresponding region in the genome [15,19–22]. The workflow takes into account frameshifts in *pncA* and *katG* as causing resistance to pyrazinamide or isoniazid, respectively. For the first-line drugs, only the mutations with known association(s) are reported to physicians for clinical considerations (an example report is provided in Supplementary Data S1).

Statistical analysis

Excel was used for data processing and statistical analysis. For the calculation of sensitivity and specificity, true and false positives were defined as strains with resistance-causing mutations which were, and were not, phenotypically resistant to the antibiotic in question, respectively. True and false negatives were defined as strains without resistance-causing mutations which were, and were not, phenotypically susceptible to the antibiotic in question, respectively.

Ethics

All data recorded in the context of the present study was not collected for research purposes but as part of the routine data collection for diagnosis. Anonymity of data was ensured before analysis.

Results

Strain selection

Between the 1st of January and 31st of December 2019, a total of 3243 cultures, sent by 78 laboratories from all over Belgium, were collected by the NRC for different purposes (species identification, DST, genotyping). Among these, 542 were identified as belonging to the *M. tuberculosis* complex and analyzed using WGS. For 306 of them, DST results were also available in the NRC (1 strain/patient, Table 1). For the remaining cultures, DST was already performed in the peripheral laboratory and the cultures were sent to the NRC for other analyses (genotyping, identification among the MTB complex, detection of resistance mutations, ...) (n = 212) or DST was impossible due to contamination (n = 18) or loss of growth (n = 6).

Phenotypic DST and conventional molecular tests

Detailed profiles of resistance to first-line drugs obtained through the phenotypic DST are provided in Supplementary Table S1 and summarised in Table 1. Of the 306 strains analysed with MGIT for first-line DST, 263 (85.9%) were susceptible to both INH and RIF.

Table 1. Description of the phenotypic and genotypic drug resistance profiles obtained on the *M. tuberculosis* complex strains analysed in 2019 at the Belgian National Reference Center.

Phenotypic (MGIT)	Genotypic (WGS)	
Drug resistance profile	Drug resistance profile	
INH-RIF-EMB-PZA	INH-RIF-EMB-PZA	Number of strains
S-S-S-S	**S-S-S-S**	233
S-S-S-R	**S-S-S-R**	12
	S-R-S-S	1
	S-S-S-S	12
S-S-S-NI	S-S-S-S	4
S-S-NI-S	S-S-S-S	1
S-R-S-S	**S-R-S-S**	1
R-S-S-S	S-S-S-S	4
	R-S-S-S	20
	R-S-R-S	2
R-S-S-R	**R-S-S-R**	2
R-R-S-S	**R-R-S-S**	4
	R-R-R-S	1
R-S-R-R	R-R-R-S	1
	R-R-R-R	1
R-R-S-R	**R-R-S-R**	1
	R-R-R-R	3
R-R-R-R	**R-R-R-R**	3
Total		306

INH: Isoniazid – RIF: Rifampicin – EMB: Ethambutol – PZA: Pyrazinamide
R: resistant; S: Susceptible; NI: Not interpretable
Bold: fully concordant between phenotypic and genotypic drug resistance profiles (for the 4 first line drugs)

Among them, 25 were phenotypically mono-resistant to PZA, including 12 isolates belonging to the *M. bovis* species which naturally harbor a resistance mutation in *pncA* [23].

Of the non-susceptible strains, 28 strains showed resistance to INH but not RIF, including two strains also presenting PZA-resistance. Only one strain presented mono-resistance to RIF. Multi-drug resistance, defined as resistance to both INH and RIF, was observed for 13 strains/patients. Noteworthy, two additional isolates (S19BD04840 and S19BD05638) should also be considered as MDR as the profile R-S-R-R was observed for respectively INH-RIF-EMB-PZA with presence of mutations in the *rpoB* gene detected by using the conventional molecular method. For one of these strains, the result DeltaWT (no match with the wild-type nor mutated probes suggesting the presence of another mutation not identifiable by the test) was obtained using the GenoType® MTBDRplus assay and for the second, a GeneXpert® MTB/RIF was performed on the culture with 'RIF resistance detected' as result.

WGS quality control

Genomic DNA (gDNA) was extracted from MGIT cultures once a week at the NRC. The amount of gDNA obtained after purification relates to density of the cultures received at the NRC, varied greatly (0.008–98.5 ng/µl), and averaged at 1.388 ng/µl (IQR 0.34 ng/µl).

A detailed analysis of samples sequenced during the first trimester of 2019 showed that 92.5% passed quality setting of the pipeline [15] directly from the first attempt, with PHRED scores ≥38 and average coverage of 104.4 ± 34.7.

Remarkably, even for sample S19BD00457 with a minimal gDNA amount of 0.008 ng/µl, high-quality sequence data could be produced (Supplementary table S1). Strains which failed QC were contaminated with host DNA or commensal bacteria, as identified by Kraken analysis on raw sequence data. The repeatability and reproducibility of the whole WGS process were evaluated previously on eight strains analyzed twice by two different technicians. The results were both 100%.

WGS data analysis

Analysis of WGS data classified all (n = 306, 100%) MTB strains correctly as *Mycobacterium tuberculosis* complex and additionally provided specific species identification within the complex.

Next, genome-based resistance profiles were generated, based on the presence of mutations and indels associated with resistance. A detailed comparison of these profiles to those obtained by phenotypic methods was performed on the 306 MTB strains on which

Table 2. Performance of whole-genome sequencing compared to phenotypic drug susceptibility testing (MGIT) for first-line drugs on the *M. tuberculosis* complex strains analysed in 2019 at the Belgian National Reference Center.

	Phenotype resistant		Phenotype Susceptible					
Drug	Genotype resistant	Genotype susceptible	Genotype resistant	Genotype susceptible	Sensitivity	Specificity	PPV	NPV
INH	38	4	0	264	90.5	100.0	100.0	98.5
RIF	12	0	3*	290	100.0	99.0	80.0	100.0
EMB	5	0	6#	294	100.0	98.0	45.4	100.0
PZA	22	14	0	266	61.1	100.0	100.0	95.0

INH: Isoniazid – RIF: Rifampicin – EMB: Ethambutol – PZA: Pyrazinamide
PPV: Positive Predictive Value
NPV: Negative Predictive Value
*_rpoB_: Leu452Pro, Leu452Pro and Asp435Tyr
#_embB_: Met306Ile (n = 2), Gly406Ser, Gly406Ala, Gln497Lys, Asp328Tyr

both WGS and MGIT DST were performed (Supplementary Table S1, Table 1). Sensitivities, specificities and positive and negative predictive values (PPV and NPV) were calculated for each first-line drug (Table 2).

Of the 263 strains that were phenotypically susceptible to both isoniazid (INH) and rifampicin (RIF), 262 were predicted to be susceptible by the bioinformatics workflows (i.e. no detection of resistance mutations). A single strain (S19BD01495) showed a mutation in *rpoB*, Asp435Tyr, known to be often missed by the MGIT system [24]. For the strain mono-resistant to RIF, the resistance profile was confirmed through WGS.

The WGS-based predictions for INH, RIF and EMB showed high sensitivity, with values of 90.5%, 100% and 100%, respectively. Only PZA showed a lower sensitivity of 61.1% caused by the 14 strains detected as PZA-resistant by the MGIT system but for which no resistance mutations were detected by WGS.

Specificities were also high for all four first-line drugs with values of 98% (EMB), 99% (RIF) and 100% (INH and PZA). The NPV was equal to or higher than 95% for the four first-line drugs. A PPV of 100% was calculated for INH and PZA and 80% for RIF. Performance was lower for ethambutol with a PPV of 45.4%, due to the detection of *embB* mutations (Met306Ile (n = 2), Gly406Ser, Gly406Ala, Gln497Lys, Asp328Tyr) in six strains that were phenotypically not associated with EMB resistance.

Two strains which were flagged as MDR-TB by WGS showed phenotypic susceptibility to rifampicin. Both predictions were based on the detection of the *rpo*B Leu452Pro mutation, which is reported by WHO as associated with a moderate level of RIF resistance and often missed with phenotypic DST like the MGIT system [24,25]. If we consider these two strains as correctly detected as RIF-resistant by WGS, the sensitivity and the PPV would both increase to 93.3%, and the specificity would increase to 99.7%.

Turnaround time (TAT)

The MTB isolates are sequenced in a centralized run of WGS which is organized by the sequencing platform of Sciensano once a week for practical and economic reasons. Depending on the day of arrival of the cultures, the TAT for WGS of MTB strains (from the reception of the cultures to reporting of results) ranges between 8 to 15 days. In comparison, the reporting of phenotypic drug resistance could take up to eight week in the conventional lab flow (Figure 1).

Discussion and conclusion

In recent years, WGS has shown the potential to revolutionize TB diagnosis and surveillance. Indeed, WGS can reliably and quickly detect the presence of mycobacteria in cultures and predict drug resistance as this is mainly provoked by genomic mutations in genes coding for drug targets or converting enzymes [5]. In the coming years, it is expected that WGS will largely replace phenotypic DST, as was already done in some countries [6,26]. Here, we have evaluated the use of WGS for species identification and detection of drug resistance in a routine NRC setting.

At the Belgian NRC, an in-house developed pipeline is used for the analysis of MTB WGS data (Bogaerts et al., 2021). When using MGIT DST as reference method, WGS showed good performance with specificities above 98% and sensitivities above 90% for all drugs except PZA (sensitivity of 61.1%). In Belgium, 85.9% of the strains received in 2019 were susceptible to INH and RIF, and 262 of these 263 strains were correctly detected by WGS as susceptible to both drugs, given the absence of detection of implicated resistance mutations. Importantly, for four strains in our 2019 collection, no causative mutations for INH resistance could be identified (Table 1). This corresponds to the 98.2% (95% CI 98.0–98.3) specificity of inferring INH resistance information from NGS data. These strains are subject of further research, and any additional identified causative mutations will be added to the database.

The continuation of phenotypic testing on isolates with no know mutations is currently under debate, although it could be seen as core task of an NRC in a high-income country.

The lower sensitivity for predicting resistance to PZA is explained by the large number of potential resistance mutations in *pnc*A and its promoter [27]. In the new WHO catalogue, group 1 mutations only had a combined sensitivity of 56.8% (95% CI, 54.8–58.8%). Therefore, the WHO added a new expert rule which says that all non-synonymous mutations in *pncA* should be assumed to confer resistance if they occur in a RIF-resistant isolate [10]. Adding to this issue, false *in vitro* resistance to PZA is known to happen with phenotypic techniques. Therefore, false phenotypic PZA resistance should be suspected especially in case of mono-resistance to PZA and a non-bovine species identification [28–31].

Both the NPV and PPV were also generally high for the evaluated drugs. Only ethambutol gave a low PPV, because many *embB* mutations confer minimal inhibitory concentrations (MICs) close to the critical concentration. This results in poor categorical agreement with phenotypic DST [29,32,33]. Moreover, it is unclear if the current breakpoint might be inflated, which could exacerbate classification of *embB* mutations as was earlier the case with borderline RIF resistance mutations [34]. This knowledge should be improved to permit the use of EMB information in routine diagnosis.

In general, our results show the effectiveness of WGS to provide reliable *M. tuberculosis* species identification and drug resistance profiles for first-line drugs in the Belgian context. As concluded by other groups, these data provide a proof of principle that phenotypic DST could be replaced by WGS for determination of resistance, and reinforce the idea to use WGS in routine workflows of TB laboratories or, at least, in NRC [4,35,36].

Thanks to the centralization at the Belgian NRC, accredited WGS diagnostics can be provided within 8 to 15 days. In contrast, conventional phenotypic DST [37] takes about 2–3 weeks in optimal conditions and requires additional weeks in case of culture contamination or failure to grow during phenotypical DST (Figure 1). Moreover, it should be noted that Belgian laboratories usually evaluate the resistance/susceptibility to first-line drugs themselves. Testing of second-line drugs is only performed in case of detection of drug resistance to first-line drugs. This provokes an additional delay of weeks before producing the final DST result for drug-resistant strains [37,38].

The time savings obtained by using the WGS is non-negligible for the detection of drug-resistant TB cases and the quick adaptation of patient treatment.

In Belgium, we observe two obstacles for the use of WGS as alternative to the phenotypical DST, i.e. (1) the absence of reimbursement of the WGS analysis by the insurance system contrary to conventional molecular analyses and (2) physicians' lack of confidence in WGS.

In Belgium, efforts have been made regarding the reimbursement of 'next generation sequencing' tests in (hematology) – oncology (Article 33 ter). A pilot study to introduce controlled NGS into the health system has been approved by the Insurance Committee in 2018 for NGS networks [39]. A multidisciplinary collaboration in NGS networks of labs and hospitals, with expertise in medical oncology, pathological anatomy, clinical biology and genome analysis has been created with NGS data registration at the national level (www.riziv.fgov.be). In medical microbiology, a similar network has been created recently for the surveillance of COVID-19 variants. To date, a parallel system for reimbursement of WGS drug resistance/susceptibility profiling of MTB strains is lacking.

Regarding the second obstacle, it remains a sound observation that the majority of medical doctors still ask phenotypical DST, as they find it difficult to rely solely on WGS-based predictions of resistance. Although the publication of a WHO catalogue of mutations [10] is an important step forwards, more studies regarding the correlation between phenotype and genotype remain needed for second line and new/repurposed anti-tuberculosis drugs.

In conclusion, WGS can provide fast and accurate drug resistance profiling, which is crucial for the adjustment of the treatment with anti-tuberculosis drugs effective on the bacteria, and subsequently, for improving treatment outcomes and reducing the subsequent transmission of resistant strains [4–6]. Our study confirms, in the Belgian context, the high performance of WGS compared to conventional phenotypic DST.

Physicians' lack of confidence in WGS and the absence of reimbursement of WGS by the insurance system are, however, two obstacles to the implementation of WGS in routine for drug resistance detection in place of phenotypic methods.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] WHO. Global tuberculosis report 2019. 2020.
[2] FARES. Registre belge de la tuberculose 2018. 2020
[3] Woldesemayat EM. Tuberculosis in migrants is among the challenges of tuberculosis control in high-income countries. RMHP. 2021;14:2965–2970.
[4] Colijn C, Cohen T. Whole-genome sequencing of Mycobacterium tuberculosis for rapid diagnostics and beyond. Lancet Respir Med. 2016;4(1):6–8.
[5] Coll F, McNerney R, Preston MD, et al. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med. 2015;7(1):51.
[6] Muzondiwa D, Mutshembele A, Pierneef RE, et al. Resistance sniffer: an online tool for prediction of drug resistance patterns of Mycobacterium tuberculosis isolates using next generation sequencing data. Int J Med Microbiol. 2020;310(2):151399.
[7] Feuerriegel S, Schleusener V, Beckert P, et al. PhyResSE: a web tool delineating Mycobacterium tuberculosis antibiotic resistance and lineage from whole-genome sequencing data. J Clin Microbiol. 2015;53(6):1908–1914.
[8] Hunt M, Bradley P, Lapierre SG, et al. Antibiotic resistance prediction for Mycobacterium tuberculosis from genome sequence data with Mykrobe. Wellcome Open Res. 2019;4:191.
[9] Steiner A, Stucki D, Coscolla M, et al. KvarQ: targeted and direct variant calling from fastq reads of bacterial genomes. BMC Genomics. 2014;15(1):881.
[10] WHO. Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance. 2021.
[11] Shea J, Halse TA, Lapierre P, et al. Comprehensive whole-genome sequencing and reporting of drug resistance profiles on clinical cases of Mycobacterium tuberculosis in New York state. J Clin Microbiol. 2017;55(6):1871–1882.
[12] Quan TP, Bawa Z, Foster D, et al. Evaluation of whole-genome sequencing for mycobacterial species identification and drug susceptibility testing in a clinical setting: a large-scale prospective assessment of performance against line probe assays and phenotyping. J Clin Microbiol. 2017;56:e01480–17.
[13] Genestet C, Hodille E, Berland J-L, et al. Whole-genome sequencing in drug susceptibility testing of Mycobacterium tuberculosis in routine practice in Lyon, France. Int J Antimicrob Agents. 2020;55(4):105912.
[14] van Beek J, Haanperä M, Smit PW, et al. Evaluation of whole genome sequencing and software tools for drug susceptibility testing of Mycobacterium tuberculosis. Clin Microbiol Infect. 2019;25(1):82–86.
[15] Bogaerts B, Delcourt T, Soetaert K, et al. A bioinformatics whole-genome sequencing workflow for clinical mycobacterium tuberculosis complex isolate analysis, validated using a reference collection extensively characterized with conventional methods and in silico approaches. J Clin Microbiol. 2021;59(6). DOI:10.1128/JCM.00202-21
[16] Bakshi CS, Shah DH, Verma R, et al. Rapid differentiation of Mycobacterium bovis and Mycobacterium tuberculosis based on a 12.7-kb fragment by a single tube multiplex-PCR. Vet Microbiol. 2005;109(3–4):211–216.
[17] Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics. 2011;27(21):2987–2993.
[18] Li H, Handsaker B, Wysoker A, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25(16):2078–2079.
[19] Coll F, Phelan J, Hill-Cawthorne GA, et al. Genome-wide analysis of multi- and extensively drug-resistant Mycobacterium tuberculosis. Nat Genet. 2018;50(2):307–316.
[20] Miotto P, Tessema B, Tagliani E, et al. A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*. Eur Respir J. 2017;50(6):1701354.
[21] Walker TM, Kohl TA, Omar SV, et al. Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. Lancet Infect Dis. 2015;15(10):1193–1202.
[22] WHO. The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex: technical guide. 2018.
[23] Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med. 1996;2(6):662–667.
[24] Van Deun A, Aung KJM, Hossain A, et al. Disputed *rpo*B mutations can frequently cause important rifampicin resistance among new tuberculosis patients. Int J Tuberc Lung Dis. 2015;19(2):185–190.
[25] Ng KCS, Ngabonziza JCS, Lempens P, et al. Bridging the TB data gap: in silico extraction of rifampicin-resistant tuberculosis diagnostic test results from whole genome sequence data. PeerJ. 2019;7:e7564.
[26] Gröschel MI, Walker TM, van der Werf TS, et al. Pathogen-based precision medicine for drug-resistant tuberculosis. PLoS Pathog. 2018;14(10):e1007297.
[27] Köser CU, Cirillo DM, Miotto P. How to optimally combine genotypic and phenotypic drug susceptibility testing methods for pyrazinamide. Antimicrob Agents Chemother. 2020;64(9):e01003–20.
[28] Chedore P, Bertucci L, Wolfe J, et al. Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to Pyrazinamide. J Clin Microbiol. 2010;48(1):300–301.
[29] Gygli SM, Keller PM, Ballif M, et al. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2019;63(4): e02175-18,/aac/63/4/AAC.02175-18.atom. DOI:10.1128/AAC.02175-18.
[30] Hoffner S, Angeby K, Sturegård E, et al. Proficiency of drug susceptibility testing of Mycobacterium tuberculosis against pyrazinamide: the Swedish experience. Int J Tuberc Lung Dis. 2013;17(11):1486–1490.

[31] Piersimoni C, Mustazzolu A, Giannoni F, et al. Prevention of false resistance results obtained in testing the susceptibility of Mycobacterium tuberculosis to Pyrazinamide with the Bactec MGIT 960 system using a reduced inoculum. J Clin Microbiol. 2013;51(1):291–294.

[32] Nonghanphithak D, Kaewprasert O, Chaiyachat P, et al. Whole-genome sequence analysis and comparisons between drug-resistance mutations and minimum inhibitory concentrations of Mycobacterium tuberculosis isolates causing M/XDR-TB. PLoS One. 2020;15(12): e0244829.

[33] Safi H, Lingaraju S, Amin A, et al. Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations in decaprenylphosphoryl-β-D-arabinose biosynthetic and utilization pathway genes. Nat Genet. 2013;45(10):1190–1197.

[34] Köser CU, Georghiou SB, Schön T, et al. On the consequences of poorly defined breakpoints for Rifampin susceptibility testing of Mycobacterium tuberculosis complex. J Clin Microbiol. 2021;59(4):e02328–20.

[35] Enkirch T, Werngren J, Groenheit R, et al. Systematic review of whole genome sequencing data to predict phenotypic drug resistance and susceptibility in Swedish *Mycobacterium tuberculosis* isolates 2016-2018. Antimicrob Agents Chemother AAC.02550-19, aac;AAC.02550-19v1. 2020. DOI:10.1128/AAC.02550-19

[36] Allix-Béguec C, Arandjelovic I, et al. CRyPTIC Consortium and the 100,000 Genomes Project. Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. N Engl J Med. 2018;379:1403–1415.

[37] Pfyffer GE, Wittwer F. Incubation time of Mycobacterial Cultures: how long is long enough to issue a final negative report to the clinician? J Clin Microbiol. 2012;50(12):4188–4189.

[38] Satta G, Atzeni A, McHugh TD. Mycobacterium tuberculosis and whole genome sequencing: a practical guide and online tools available for the clinical microbiologist. Clin Microbiol Infect. 2017;23 (2):69–72.

[39] Van Valckenborgh E, Hébrant A, Antoniou A, et al. Roadbook for the implementation of next-generation sequencing in clinical practice in oncology and hemato-oncology in Belgium. Arch Public Health. 2018;76(1):49.